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Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans

Marco

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Nauck, Michael A., Ulrich Niedereichholz, Rainer Ettler, Jens Juul Holst, Cathrine Ørskov, Robert Ritzel, and Wolff H. Schmiegel. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am. J. Physiol.* 273 (*Endocrinol. Metab.* 36): E981–E988, 1997.—Glucagon-like peptide 1 (GLP-1) has been shown to inhibit gastric emptying of liquid meals in type 2 diabetic patients. It was the aim of the present study to compare the action of physiological and pharmacological doses of intravenous GLP-1-(7—36) amide and GLP-1-(7—37) on gastric emptying in normal volunteers. Nine healthy subjects participated (26 ± 3 yr; body mass index 22.9 ± 1.6 kg/m^2; hemoglobin A_{1c} 5.0 ± 0.2%) in five experiments on separate occasions after an overnight fast. A nasogastric tube was positioned for the determination of gastric volume by use of a dye-dilution technique (phenol red). GLP-1-(7—36) amide (0.4, 0.8, or 1.2 pmol·kg^{-1}·min^{-1}), GLP-1-(7—37) (1.2 pmol·kg^{-1}·min^{-1}), or placebo was infused intravenously from −30 to 240 min. A liquid meal (50 g sucrose, 8% amino acids, 440 ml, 327 kcal) was administered at 0 min. Glucose, insulin, and C-peptide were measured over 240 min. Gastric emptying was dose dependently slowed by GLP-1-(7—36) amide ($P < 0.0001$). Effects of GLP-1-(7—37) at 1.2 pmol·kg^{-1}·min^{-1} were virtually identical. GLP-1 dose dependently stimulated fasting insulin secretion (−30 to 0 min) and slightly reduced glucose concentrations. After the meal (0–240 min), integrated incremental glucose ($P < 0.0001$) and insulin responses ($P = 0.01$) were reduced (dose dependently) rather than enhanced. In conclusion, *1*) GLP-1-(7—36) amide or -(7—37) inhibits gastric emptying also in normal subjects, *2*) physiological doses (0.4 pmol·kg^{-1}·min^{-1}) still have a significant effect, *3*) despite the known insulinotropic actions of GLP-1-(7—36) amide and -(7—37), the net effect of administering GLP-1 with a meal is no change or a reduction in meal-related insulin responses. These findings suggest a primarily inhibitory function for GLP-1 (ileal brake mechanisms).

incretin hormones; glucagon-like peptide 1-(7—36) amide; pancreatic glucagon; enteroinsular axis

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THE EXISTENCE OF GLUCAGON-LIKE PEPTIDE 1 [GLP-1; amino acid sequence (1—37)] was predicted on the basis of analysis of the proglucagon gene (1). Later, it became clear that GLP-1-(7—36) amide and, to a lesser degree, GLP-1-(7—37), are produced in L cells in the lower gastrointestinal tract (6, 25). Synthetic GLP-1-(7—36) amide and -(7—37) stimulate insulin secretion in the perfused pancreas (24) and, when infused into humans, in both normal glucose-tolerant (15, 19) and type 2 diabetic (non-insulin-dependent diabetic) subjects (9, 18, 21), especially at elevated glucose concentrations [glucose dependence (15, 18, 19, 21)]. As an insulinotropic agent, GLP-1 appears to be the pharmacologically more potent counterpart to gastric inhibitory polypeptide [GIP (15, 19)]. GLP-1, therefore, seems to be the long-sought second incretin hormone, explaining the phenomenon that oral glucose elicits a greater insulin secretory response than is explained by the rise in glycemia alone (2).

An incretin role for GLP-1 would imply that physiological increments of GLP-1 plasma concentrations are accompanied by evidence of stimulated insulin secretion during the postprandial phase of physiological hyperglycemia. This has been demonstrated only in animal experiments by use of a specific GLP-1 receptor antagonist, exendin-(9—39), which blocked insulin secretory responses when glucose was administered orally (29) or intraduodenally (14) in rats. In these experiments, another potent action of GLP-1, the deceleration of gastric emptying (30, 31), was not studied and/or had little impact. This inhibitory action of GLP-1 on gastric emptying has been described in normal subjects (30) and in type 2 diabetic patients (31). In type 2 diabetic patients with hyperglycemia, exogenous GLP-1 in a pharmacological dose (1.2 pmol·kg^{-1}·min^{-1}) nevertheless stimulated insulin secretion and inhibited glucagon secretion despite a near-complete standstill of gastric emptying (31). In normal subjects, the prevention of the duodenal delivery of nutrients would interfere with the rise in glycemia that amplifies the synergistic insulinotropic actions of GLP-1 (15, 19).

Therefore, we wanted to study the effects of an exogenous administration of GLP-1-(7—36) amide in healthy volunteers fed a liquid mixed meal under conditions that allowed the measurement of gastric emptying by use of a dye-dilution technique (phenol red). The dosage of GLP-1 was chosen to span the physiological and pharmacological range as defined by previous studies (15, 19, 31). Furthermore, the actions of GLP-1-(7—36) amide were compared with those of GLP-1-(7—37) (25, 26). It was our aim to elucidate *1*) whether the inhibition of gastric emptying by GLP-1 is dose dependent and whether it occurs also with physiological increments in GLP-1 plasma concentrations, *2*) whether GLP-1-(7—36) amide and -(7—37) might differ with respect to their gastric actions, and *3*) whether, under these conditions, the net effect of exogenous GLP-1 is an augmentation of or a reduction in meal-related insulin secretory responses, i.e., if the effects on gastric emptying would potentially outweigh the insulinotropic effects. Furthermore, these questions have to be answered to define the GLP-1 plasma concentration range compatible with normal nutrition in type 2

diabetic patients, in whom inhibition of gastric emptying (31) may question a potential "therapeutic" use of GLP-1 or of its derivatives that has been suggested on the basis of its preserved insulinotropic (18, 21) and glucagon-lowering (21) actions in this patient group. Preliminary results have been published in abstract form (20).

SUBJECTS, MATERIALS, AND METHODS

Study protocol. The study protocol was approved by the ethics committee of the medical faculty of Ruhr-University, Bochum, on October 21, 1993 (registration number 437) before the study. Written informed consent was obtained from all participants.

Subjects. Nine healthy volunteers were studied. They were 26 ± 3 yr old, were 187 ± 7 cm tall, weighed 79 ± 5 kg (body mass index was 22.9 ± 1.6 kg/m^2), and their hemoglobin A_{1C} was 5.0 ± 0.2% (normal range, 4.0–6.2%). All had a normal oral glucose tolerance according to World Health Organization criteria (fasting glucose 4.5 ± 0.4, 120-min value 4.4 ± 0.8 mmol/l). None had a family history of diabetes mellitus or a personal history of gastrointestinal disorders. Blood cell counts, serum transaminases, creatinine values, and triglyceride, total cholesterol, and high-density cholesterol concentrations were in the normal range.

Study design. All participants were studied, in random order, on five occasions. *1)* A liquid mixed meal (50 g sucrose plus amino acids, 400 ml Aminosteril N-Hepa 8%; Fresenius, Bad Homburg, Germany) was instilled intragastrically at *time 0*. Placebo (0.9% NaCl with 1% human serum albumin, Human Albumin 20% Behring, salzarm, Behringwerke, Marburg, Germany) was infused intravenously from −30 to 240 min. *2)* A liquid meal was instilled intragastrically at *time 0*. GLP-1-(7—36) amide, 0.4 pmol·kg^{-1}·min^{-1}, was infused intravenously from −30 to 240 min. *3)* A liquid meal was instilled intragastrically at *time 0*. GLP-1-(7—36) amide, 0.8 pmol·kg^{-1}·min^{-1}, was infused intravenously from −30 to 240 min. *4)* A liquid meal was instilled intragastrically at *time 0*. GLP-1-(7—36) amide, 1.2 pmol·kg^{-1}·min^{-1}, was infused intravenously from −30 to 240 min. *5)* A liquid meal was instilled intragastrically at *time 0*. GLP-1-(7—37), 1.2 pmol·kg^{-1}·min^{-1}, was infused intravenously from −30 to 240 min. All volunteers were studied at intervals of 7–10 days. A single experiment was performed on one volunteer per day.

Peptides. Synthetic GLP-1-(7—36) amide and GLP-1-(7—37) were purchased from Saxon Biochemicals (Hannover, Germany). The lot number of GLP-1-(7—36) amide (pharmaceutical grade) was PGAS 242, FGLP7369301 A, and net peptide content was 88%. The lot number for GLP-1-(7—37) was PGAS 243, Lot ZJ 222, and net peptide content was 91%. The peptides were dissolved in 0.9% NaCl/1% human serum albumin, filtered through 0.2-μm nitrocellulose filters (Millipore, Bedford, MA), and stored frozen at −30°C as previously described. High-performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was >99% pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and for pyrogens (Limulus amebocyte lysate endo-LAL, Chromogenix, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1 stock solutions (50 μg peptide/ml) always were <0.08 endotoxin units/ml.

Experimental procedures. The tests were performed in the morning after an overnight fast. Two forearm veins were punctured with an 18-gauge Teflon cannula (Moskito 128, Vygon, Aachen, Germany), and the cannulas were kept patent with 0.9% NaCl for blood sampling and for GLP-1 and/or placebo administration.

After basal blood specimens were drawn, at −30 min an intravenous infusion of GLP-1-(7—36) amide or -(7—37) or placebo (0.9% NaCl containing 1% human serum albumin) was started and continued for 270 min. The infusion rates were based on previous studies (15, 19) and were selected to raise plasma GLP-1 concentrations into the upper physiological (15, 19) to pharmacological range (9, 18, 21) [~2- to 4-fold higher concentrations than those measured after oral nutrients (15, 19, 31)]. The infusion was begun at −30 min to assure elevated GLP-1-(7—36) amide plasma concentrations already at the time point of administration of the liquid meal. Blood was drawn at the time points indicated in Figs. 3 and 4, and plasma glucose was determined immediately.

Gastric emptying. Before the study, a 120-cm nasogastric tube (CH12, Freka-Ernährungssonde, Fresenius) was placed and tape-fixed with the tip ~55 cm from the nostrils. Gastric juice was aspirated, and an acidic pH was ascertained with pH-sensitive Lackmus paper. The gastric lumen was washed with 100 ml of water (37°C). The position of the tube was adjusted to allow a near-complete aspiration of instilled fluid. The subjects lay on their backs in a semi-recumbent position, with the upper half of the body 45° upright. At 0 min, 440 ml (total volume) of the liquid test meal was instilled into their stomachs. It was composed of 50 g of sucrose dissolved in 400 ml of Aminosteril Hepa 8% (Fresenius). The amino acid content of the meal (in mmol) was 131.7 isoleucine, 39.9 leucine, 18.8 lysine, 2.9 methionine, 1.7 cysteine, 31.0 glycine, 2.1 phenylalanin, 14.8 threonine, 1.4 tryptophan, 34.4 valine, 20.4 arginine, 7.2 histidine, 20.8 alanine, 19.9 proline, and 8.5 serine. Acetic acid (29.4 mmol) was also contained in this commercial amino acid mixture.

This composition of the meal was chosen because the solution had to be clear for the photometric measurement of phenol red (see description of the measurement of gastric emptying to follow) and should have been similar in caloric and nutrient content to a normal mixed meal. Amino acids were added to stimulate the release of cholecystokinin (17), a physiological regulator of gastric emptying in humans. The meal contained 32 g of mixed amino acids (131 kcal = 40%) and 50 g of sucrose (196 kcal = 60%) (20), and total energy content was 327 kcal (energy density: 0.82 kcal/ml).

Gastric emptying was measured by a double-sampling dye-dilution technique using phenol red (Merck, Darmstadt, Germany) according to George (7), with modifications introduced to reduce measurement error by Hurwitz (12). In principle, at all time points chosen to measure gastric volume, a known amount of the nonabsorbable phenol red dye was added to the translucent liquid test meal in a volume of 5 to 15 ml. After thorough mixing with gastric contents for ~2 min, a gastric sample was drawn, and the resulting step-up in phenol red concentrations was determined photometrically. The volume of gastric contents was determined as the volume of distribution of phenol red. Increasing amounts of phenol red were used as the experiments proceeded to get well-measurable increments in optical density also in the presence of previously instilled phenol red. According to the expected rate of gastric emptying (30, 31), gastric contents were determined at intervals (see Fig. 2) over 240 min.

Blood specimens. Blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 20,000 kallikrein-inhibitor units/ml, 200 μl/10 ml blood; Bayer, Leverkusen) and kept on ice. A sample (~100 μl) was stored in NaF (Microvette CB 800; Sarstedt, Nümbrecht, Germany) for the measurement of glucose. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −30°C.

Laboratory determinations. Glucose was measured using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany). Insulin was measured using an insulin microparticle enzyme immunoassay, IMx Insulin (Abbott Laboratories, Wiesbaden, Germany). Intra-assay coefficients of variation were ≈4%. C-peptide was measured using C-peptide antibody-coated microtitre wells (C-peptide MTPL EIA) from DRG Instruments (Marburg, Germany). Intra-assay coefficients of variation were ≈6%. Human insulin and C-peptide were used as standards.

GLP-1 immunoreactivity was determined in ethanol-extracted plasma (final concentration, 70%) after freeze-drying and resuspension in assay buffer, as previously described (23), by use of antiserum 89390 (final dilution 1:150,000) for the measurement of GLP-1-(7—36) amide and synthetic GLP-1-(7—36) amide for tracer preparation and as standard. Recovery of GLP-1-(7—36) amide standards (320 pmol/l) after alcohol extraction was 75 ± 8%. Similar recoveries were seen with GLP-1-(7—37). The experimental detection limit [2 SDs over samples not containing GLP-1-(7—36) amide] was <7 pmol/l. Approximately 10–14 pmol/l (3–4.2 fmol/sample ≈ 300 μl) displaced 50% of labeled GLP-1-(7—36) amide. Antiserum 89390 binds to the amidated carboxy terminus of GLP-1-(7—36) amide (25, 26). When GLP-1-(7—37) was infused, measurements were performed with antiserum 2135 (final dilution 1:150,000). This antibody binds to the midportion of GLP-1 and reacts with equal affinity with both GLP-1-(7—36) amide and -(7—37) (25, 26). The experimental detection limit was <5 pmol/l. Approximately 28 pmol/l (8.4 fmol/sample) displaced 50% of labeled GLP-1-(7—36) amide or -(7—37). GLP-1-(7—37) values were normalized for the concentrations measured with antiserum 2135 during infusion of the highest dose of GLP-1-(7—36) amide, 1.2 pmol·kg^{-1}·min^{-1}, which were measured using both antisera 89390 and 2135. Intra-assay coefficients of variation were ≈8%.

Pancreatic glucagon was assayed in ethanol-extracted plasma (same procedure as described for GLP-1, recovery 80 ± 10%) by use of antibody 4305 (10). The detection limit is ~1 pmol/l, and the intra-assay coefficient of variation is <6% in the working range (half-maximal displacement of labeled glucagon at ~14 pmol/l).

Phenol red in gastric contents was assayed photometrically after filtration through filter paper (100 μl in 2 ml Na_2HPO_4/NaH_2PO_4 buffer, 0.6 mol/l, pH 8.0) at a wavelength of 546 nm and read against a standard curve (phenol red in phosphate buffer).

Chloride in samples of gastric content was measured using a chloride meter (Ciba-Corning, Munich, Germany).

Each patient's set of plasma samples was assayed at the same time to avoid errors due to interassay variation.

Statistical analysis. Results are reported as means ± SE. Integrations were carried out according to the trapezoidal rule, with separate calculations for increments above and decrements below baseline. Meal-related responses were calculated over true baseline values (as determined at −45/−30 min of Figs. 2 and 3), because GLP-1 administration changed values determined at 0 min.

Insulin secretory rates were calculated using "deconvolution" analysis and a two-compartment model for C-peptide kinetics described by Eaton et al. (4) and Polonsky et al. (27). The software ISEC (version 2.0) was kindly provided by Dr. R. Hovorka, Centre for Measurement and Information in Medicine, Department of Systems Science, City University, London, UK (11). This software uses standard kinetic rate constants (k_1, k_2, and k_3) for the transition of C-peptide between compartments or the loss of C-peptide from *compartment 1* based on age, gender, body length, and weight (11).

Chloride output was estimated for each sampling interval and related to the geometric mean of gastric volume measured at the beginning and end of each period.

All statistical calculations were carried out using repeated-measures analysis of variance (ANOVA) with NCSS Version 5.01 (Jerry Hintze, Kaysville, UT). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by one-way ANOVA, followed by Student's *t*-test (paired analyses) if P values were <0.05. P values were corrected for the number of comparisons made, according to Bonferroni-Holm. A corrected two-sided P value of <0.05 was taken to indicate significant differences.

RESULTS

Plasma GLP-1 concentrations. The liquid mixed meal raised plasma GLP-1-(7—36) amide concentrations from basal ≈ 5 pmol/l to 11.6 ± 1.6 pmol/l at 30 min, with a return to baseline values at ~150 min. Steady-state plasma concentrations during the exogenous administration of GLP-1-(7—36) amide were ≈ 25, 36, and 51 pmol/l for doses of 0.4, 0.8, and 1.2 pmol·kg^{-1}·min^{-1} reached 15–60 min after start of the infusion (Table 1). In any case, clearly elevated concentrations were already achieved at the time point of liquid test meal administration (Fig. 1, *top*). Plasma levels during the infusion of GLP-1-(7—37) were comparable to those obtained with the same dose of GLP-1-(7—36) amide. Directly measured steady-state concentrations as determined using the nonspecific antiserum 2135 were 87 ± 10 pmol/l [GLP-1-(7—37)] and 93 ± 6 pmol/l [GLP-1-(7—37)].

Gastric emptying. With placebo, gastric volume declined over 120 min to a residual value of 54 ± 18 ml, with half-emptying occurring at 45–60 min. Under the influence of exogenous GLP-1-(7—36) amide, gastric emptying was dose dependently decelerated (Fig. 2). Even the lowest dose studied, 0.4 pmol·kg^{-1}·min^{-1}, significantly slowed gastric emptying. Effects of GLP-1-(7—37) were similar to those of GLP-1-(7—36) amide. With the highest dose, gastric contents remained at ≈250 ml 240 min after the meal was instilled.

Plasma glucose. The liquid test meal caused an increment in plasma glucose with placebo (Fig. 3, *top*).

Table 1. *GLP-1 steady-state concentrations and premeal glucagon responses during iv GLP-1-(7—36) amide or -(7—37)*

GLP-1 Molecular Form	Dose, pmol·kg^{-1}·min^{-1}	Steady-State GLP-1 Concentration, pmol/l	Preprandial Glucagon Responses, pmol·l^{-1}·min^{-1}
Placebo	0	7 ± 1	−19 ± 6
(7—36) Amide	0.4	25 ± 3*	−46 ± 7*
(7—36) Amide	0.8	36 ± 3*	−69 ± 8*
(7—36) Amide	1.2	51 ± 4*	−72 ± 17*
(7—37)	1.2	47 ± 6*	−68 ± 16*
Direction (from baseline)			(negative)
P (ANOVA)		0.0001	0.012

Values are means ± SE; $n = 9$ subjects. Glucagon responses were determined in basal state before ig instillation of a liquid mixed meal. GLP-1, glucagon-like peptide 1. *Significantly different ($P < 0.05$ by *t*-test) from placebo.

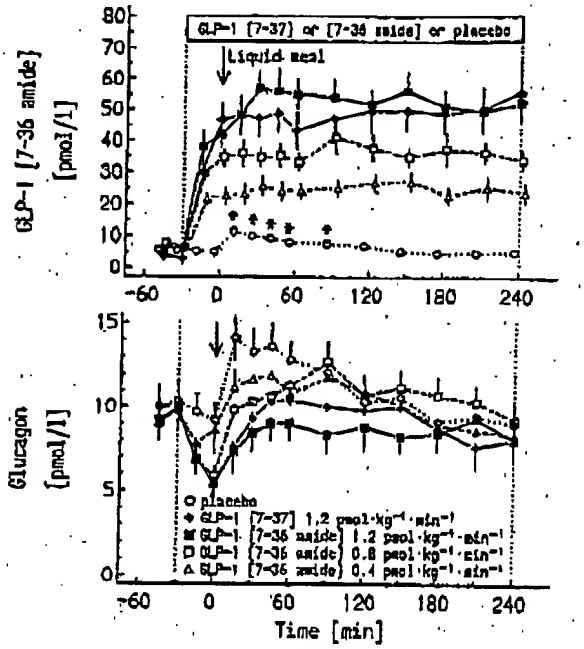


Fig. 1. Plasma concentrations of glucagon-like peptide 1 (GLP-1, *top*) and glucagon (*bottom*) (means ± SE) during iv infusion of GLP-1-(7—36) amide or -(7—37); symbols show different doses in 9 healthy male volunteers. * Significantly different ($P < 0.05$ by paired *t*-test) increments vs. baseline values with iv placebo. Box, duration of infusion of GLP-1/placebo.

With GLP-1-(7—36) amide or -(7—37), glucose concentrations started to decline when the exogenous (intravenous) administration was initiated. Also, meal-related increments in glycemia were reduced or abolished,

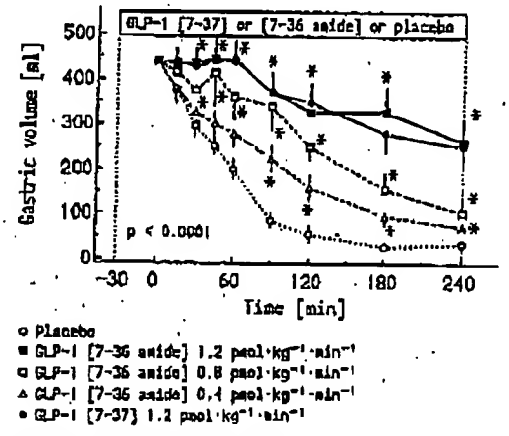
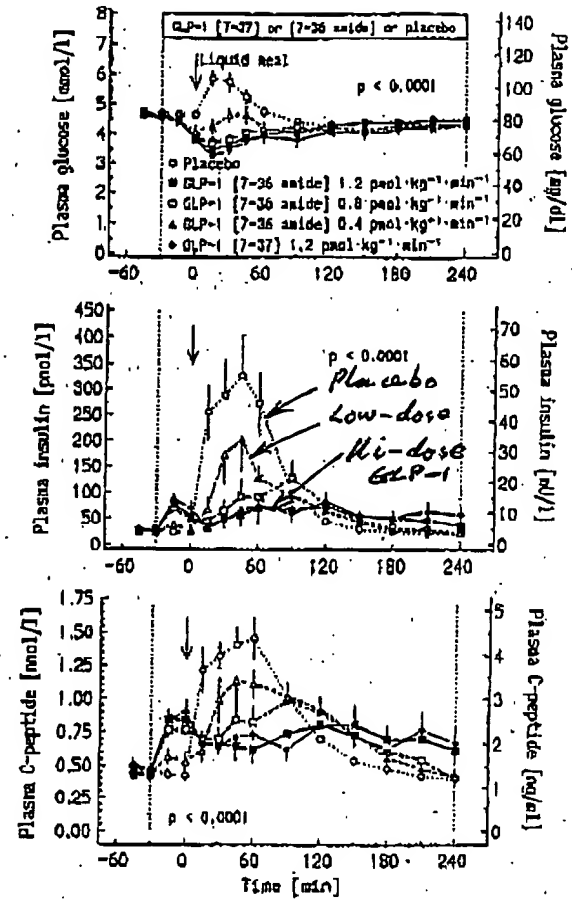


Fig. 2. Residual gastric volume after mixed liquid meal (8% amino acids plus 50 g sucrose in 400 ml) during iv infusion of GLP-1-(7—36) amide or -(7—37) (means ± SE); symbols show different doses in 9 healthy male volunteers. *P* values represent interaction of experiment (placebo/GLP-1) and time as calculated by repeated-measures analysis of variance (RM-ANOVA). * Significant differences ($P < 0.05$ by Student's *t*-test) from experiments with placebo at individual time points. Box, duration of infusion of GLP-1/placebo.



Insulin

Fig. 3. Plasma concentrations of glucose (*top*), insulin (*middle*), and C-peptide (*bottom*) (means ± SE) during iv infusion of GLP-1-(7—36) amide or -(7—37); symbols show different doses in 9 healthy male volunteers. *P* values represent interaction of experiment (placebo/GLP-1) and time as calculated by RM-ANOVA. Box, duration of infusion of GLP-1/placebo.

respectively, depending on the dose of GLP-1 administered (Fig. 3).

Insulin secretory responses. Insulin and C-peptide increased during the infusion of GLP-1-(7—36) amide (starting at -30 min) or, in the placebo experiment, after the liquid meal was instilled (0 min) (Fig. 3). Meal-related increments in insulin and C-peptide (from 29 ± 2 to 325 ± 78 pmol/l at 45 min and from 0.42 ± 0.04 to 1.45 ± 0.16 nmol/l at 60 min, respectively, with placebo), however, were reduced rather than enhanced by the simultaneous administration of GLP-1. GLP-1-(7—37) was as effective as GLP-1-(7—36) amide. This reduction in meal-related integrated increments (Table 2) was significant in the case of insulin responses ($P = 0.01$) but not for C-peptide measurements ($P = 0.19$). However, C-peptide responses clearly did not increase because of the administration of GLP-1. Both insulin and C-peptide tended to remain elevated by the end of the experiments with the highest doses of GLP-1-(7—36)

Table 2. *Integrated incremental responses over baseline of glucose, insulin, and C-peptide*

	Placebo	GLP-1-(7—36) Amide, pmol·kg^{-1}·min^{-1}			GLP-1-(7—37), pmol·kg^{-1}·min^{-1}	Significance of Difference, *P* (ANOVA)
		0.4	0.8	1.2	1.2	
Glucose, mmol·l^{-1}·min^{-1}	51.1 ± 7.7	8.9 ± 4.4*	4.2 ± 3.4*	4.0 ± 2.9*	8.4 ± 6.2*	<0.0001
Insulin						
Preprandial, pmol·l^{-1}·min^{-1}	2.4 ± 1.8	252 ± 53*	783 ± 97*	1013 ± 150*	1194 ± 208	<0.0001
Postprandial, nmol·l^{-1}·min^{-1}	20.1 ± 4.3	12.0 ± 2.4*	8.5 ± 2.1	7.3 ± 1.8*	8.0 ± 2.2*	0.01
C-peptide, pmol·l^{-1}·min^{-1}						
Preprandial	0.1 ± 0.1	2.8 ± 0.7*	6.8 ± 0.5*	9.2 ± 0.7*	8.3 ± 1.5*	<0.0001
Postprandial	82.9 ± 7.4	84.0 ± 10.2	65.6 ± 11.9	64.7 ± 13.9	51.8 ± 9.2	0.19

Values are means ± SE; $n = 9$ subjects. Glucose values are total responses from −30 to 240 min; those of insulin are either from −30 to 0 min (preprandial) or from 0 to 240 min over baseline at −45/−30 min (postprandial). *Significant difference ($P < 0.05$ by paired *t*-test) from placebo.

amide and -(7—37) but returned to basal levels with 0.4 and 0.8 pmol·kg^{-1}·min^{-1} GLP-1-(7—36) amide (Fig. 3). Integrated insulin secretory responses (Fig. 4) also showed a preprandial stimulation but a trend to a postprandial reduction due to GLP-1 ($P = 0.10$).

Pancreatic glucagon. In the placebo study, there was a clear meal-related response of glucagon (Fig. 1, *bottom*). Exogenous GLP-1 dose dependently reduced glucagon before the liquid test meal was instilled (−30 to 0 min) and blunted meal-related increments.

Symptoms. In some volunteers, abdominal discomfort was reported during the high-dose GLP-1 infusions, typically at time points when phenol red was mixed with gastric contents.

Gastric chloride secretion. Chloride output increased after the intragastric instillation of the liquid meal (Fig. 5). A rapid increment was followed by a more sustained stimulation. With GLP-1, the early chloride output was reduced. This, however, was significant only for the lowest dose ($P = 0.02$). Later in the time course, chloride output was enhanced by GLP-1. At these time points, more gastric content was present when GLP-1 was administered (Fig. 2).

DISCUSSION

The physiological incretin role for GLP-1 was deduced from experiments showing glucose-dependent stimulation of insulin secretion in perfused pancreas models (24) and in humans (15, 19), to whom glucose was administered together with GLP-1 in doses leading to close-to-physiological plasma concentrations. More recently, insulin responses to oral (29) or intraduodenal (14) glucose in rats were shown to be reduced by a specific GLP-1 receptor antagonist, exendin-(9—39). Experiments in humans to explore effects of exogenous GLP-1 administered together with a meal have been reported only in type 2 diabetic patients (31) or have not specifically examined the consequences of decelerated gastric emptying on postprandial glucose, insulin, and glucagon responses (3, 8, 9, 18).

The present results demonstrate that GLP-1-(7—36) amide and -(7—37) have a similar, profoundly inhibitory effect on gastric emptying of a liquid mixed test meal in healthy, normoglycemic volunteers (Fig. 2) and that the effect of GLP-1 on gastric emptying is dose dependent and highly significant also, with physiological

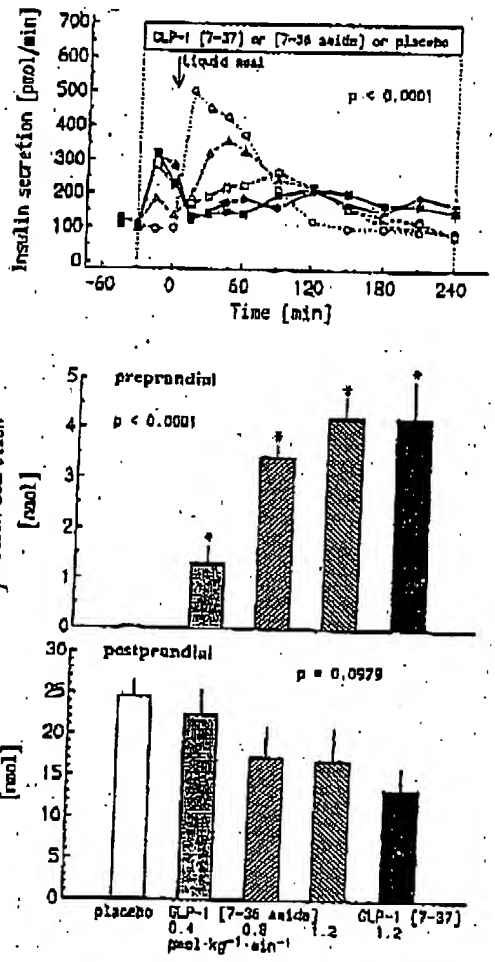


Fig. 4. Insulin secretion rates (*top*) and integrated incremental insulin secretory responses during preprandial (−30 to 0 min; *middle*) and postprandial (0–240 min; *bottom*) periods during iv infusion of different doses of GLP-1-(7—36) amide or -(7—37). Values are means of data from 9 healthy male volunteers; SEs were up to 15% of mean values (not shown). Symbols are as in Figs. 1–3. *P* values represent interaction of experiment (placebo/GLP-1) and time as calculated by RM-ANOVA or one-way ANOVA. *Significant differences ($P < 0.05$ by Student's *t*-test) from experiments with placebo.

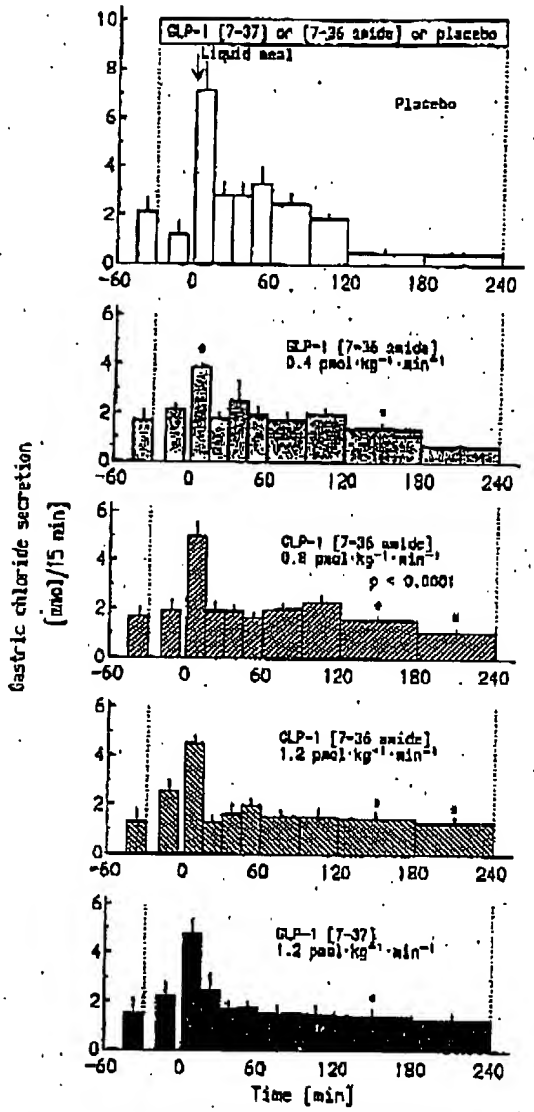


Fig. 5. Gastric chloride output after mixed liquid meal (8% amino acids plus 50 g sucrose in 400 ml) during iv infusion of different doses of GLP-1-(7—36) amide or -(7—37); values are means ± SE of 9 healthy male volunteers. *P* values represent interaction of experiment (placebo/GLP-1) and time as calculated by RM-ANOVA. *Significant differences ($P < 0.05$ by Student's *t*-test) from experiments with placebo at individual time points. Box, duration of infusion of GLP-1/placebo.

concentrations ~25 pmol/l (Figs. 1 and 2). In the present study GLP-1-(7—36) amide concentrations after intragastric instillation of a liquid test meal were only ~12 pmol/l, but in previous experiments involving normal mixed meals, but using the same assay methodology [antiserum 89390 (25, 26)], postprandial GLP-1-(7—36) amide concentrations were stimulated to 21 ± 4 pmol/l with the same test meal in type 2 diabetic patients (31). This shows that the plasma level of GLP-1 achieved by the lowest infusion rate of GLP-1-(7—36) amide (0.4 pmol·kg^{-1}·min^{-1}), in line with previous studies (15, 19), is similar to approximately postprandial increments in GLP-1-(7—36) amide plasma concentrations. Therefore, like cholecystokinin (17), GLP-1-(7—36) amide seems to effectively decelerate gastric emptying even at physiological plasma concentrations.

Because the dye-dilution technique used in the present experiments measures only gastric volume and not duodenal delivery, differences in intragastric volume curves could be the result of differences in gastric juice secretion during the experiments. GLP-1 has been shown to reduce pentagastrin-stimulated gastric acid secretion (22, 28). A priori, increasing doses of GLP-1 would be expected to decrease intragastric volume rather than the opposite. Therefore, the differences in the gastric volume (Fig. 2) can mainly be ascribed to effects on gastric emptying, which, if anything, are likely to be somewhat underestimated. On the basis of chloride secretion rates (Fig. 5), there were only marginal changes in gastric acid secretion due to GLP-1 administration. Therefore, the differences in gastric volume observed (Fig. 2) can mainly be ascribed to effects on gastric emptying itself.

In contrast to experiments with subcutaneous administrations of GLP-1, which lead to a short-lived elevation of GLP-1 plasma levels (8), the deceleration of gastric emptying with intravenous GLP-1 lasts as long as plasma GLP-1 concentrations are elevated, both in type 2 diabetic patients (31) and in healthy subjects (this study).

In line with previous studies (15, 19), there was a significant and dose-related effect of exogenous GLP-1 on insulin (Figs. 3 and 4) and glucagon (Fig. 1, *bottom*) concentrations in fasting normal subjects, leading to a reduction in fasting glycemia. Again, this produced no clinical hypoglycemia, even at pharmacological doses. Surprisingly, however, postprandial insulin and glucagon responses were unchanged or diminished by the administration of GLP-1, and not enhanced, as would be expected for an incretin hormone (2). This is most likely explained by the inhibition of gastric emptying (Fig. 2), which slows the transit of nutrients into the duodenum and jejunum, where the absorption of glucose, fructose, and amino acids takes place, as is clearly illustrated by plasma glucose concentrations that remained below basal levels in all experiments involving exogenous GLP-1 (15, 19). Because the mechanism of GLP-1's insulinotropic effect is to potentiate substrate-induced secretion, it is understandable that the insulin response decreased despite increased GLP-1 concentrations.

If one accepts the conditions of this study (a liquid mixed meal) as physiological, the results challenge a physiological incretin role for GLP-1. Because there are differences in the regulation of gastric emptying of liquid and solid meal components, which may be differentially affected by agents that regulate the velocity of emptying, the present results should be reproduced with a meal also containing solid components. However, in the study by Gutniak et al. (9), even with a

normal test meal, reduced meal-related insulin and glucagon responses were described in normal volunteers receiving an intravenous infusion of 0.75 pmol·kg^{-1}·min^{-1} GLP-1-(7—36) amide. In their study, gastric emptying was not measured directly.

Because with nutrients administered into the lower small intestinal lumen inhibitory effects on upper gastrointestinal functions [e.g., gastric emptying, antroduodenal motility, exocrine pancreatic secretion (16)] have been documented, the main physiological role for GLP-1 may be participation in the "ileal brake" mechanism (16). This would also be more compatible with its main localization, the ileum and colon/rectum (6). The importance of the other incretin hormone, GIP, would then be strengthened by the present results (15, 19). GIP, in contrast to GLP-1, does not inhibit but rather accelerates gastric emptying slightly (5). Furthermore, physiological replacement doses of GIP in healthy human subjects almost reproduce insulin secretory responses during physiological hyperglycemia to levels observed after oral glucose stimulation (19).

There has been concern about the almost total inhibition of gastric emptying caused by exogenous GLP-1-(7—36) amide (1.2 pmol·kg^{-1}·min^{-1}) in type 2 diabetic patients (31). It could be extrapolated that, during attempts to reduce glycemia through the insulinotropic and glucagonostatic actions of GLP-1, nutritional problems or side effects could be caused by the inhibitory actions of GLP-1 on gastric emptying if pharmacological concentrations are maintained throughout the day. Th present results show that a reduction in therapeutic plasma levels to those achieved by an infusion rate of 0.8 pmol·kg^{-1}·min^{-1} leads to nearly complete gastric emptying after 240 min (Fig. 2). Because 1.0 to 1.2 pmol·kg^{-1}·min^{-1} GLP-1-(7—36) amide or -(7—37) infused intravenously completely normalized fasting hyperglycemia in type 2 diabetic patients within 3–4 h in recent studies (21, 31), a slight reduction in dosage to 0.8 pmol·kg^{-1}·min^{-1} can still be assumed to be effective. It may be suggested that the partial inhibition of gastric emptying observed with this dose may add to the therapeutic effectiveness of GLP-1, because slowing nutrient entry into the circulation by dietary measures or α-glucosidase inhibition is an established principle in the therapy of type 2 diabetic patients.

In conclusion, the present study demonstrates *1*) a dose-dependent inhibitory effect on gastric emptying of a liquid mixed meal by exogenous GLP-1 in healthy volunteers that was similar in magnitude for GLP-1-(7—36) amide and -(7—37). *2*) At all GLP-1 plasma concentrations studied, including a physiological replacement dose, meal-related insulin secretory responses were diminished rather than enhanced, emphasizing GLP-1's functions as one of the hormones of the ileal brake mechanism. *3*) It should be carefully studied whether GLP-1 may be administered as a therapeutic agent in type 2 diabetic patients during meal ingestion. It must be made certain that the dosage and plasma levels are in the range that leads to only a partial inhibition of gastric emptying.

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